CELL NUMBER IN RELATION TO SEED SIZE IN PEANUT (*Arachis hypogaea* L.)

BY

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A DISSERTATION PRESENTED TO THE GRADUATE COUNCIL
OF THE UNIVERSITY OF FLORIDA IN
PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

1981

ACKNOWLEDGEMENTS

I would like to thank Dr. F.P. Gardner for being chairman of my supervisory committee and for the competent guidance during the latter part of my research program. I gained a very valuable experience working with him. I wish to express my sincere appreciation to Dr. D.E. McCloud for being the chairman of my supervisory committee earlier in the graduate program. I also express my appreciation to the following members of my supervisory committee: Dr. W.G. Duncan, Dr. A.J. Norden, Dr. K.H. Quesenberry and Dr. W.G. Blue. Special thanks are due Dr. Duncan for his extensive assistance and helpful criticism in the preparation of this study.

Thanks are also in order to all the graduate students who have been helpful and to Mr. R.A. Hill and Mrs. Beverly Barbarette for their assistance.

I also gratefully acknowledge Dr. D.B. Egli, Agronomy Department, University of Kentucky, for giving information on the cell measurement technique; Drs. S.C. Schank, K.H. Quesenberry and M.H. Gaskins for their help with the laboratory procedures; Dr. K.J. Boote for reviewing the dissertation and making very valuable suggestions; and Dr. R.C. Littell for his help with statistical analysis.

I am deeply grateful for the understanding and encouragement of my husband Anil.

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Abstract of Dissertation Presented to the Graduate Council of the University of Florida in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy

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August 1981

Chairman: Franklin P. Gardner
Major Department: Agronomy

Greenhouse studies were conducted on peanuts (Arachis hypogaea L.) with five different cultivars: 'Florigiant', 'Florunner', 'Starr', 'Krinkle' and 'Chico'. The objective of the study was to relate the cell number in the seeds of these cultivars to seed size and seed growth rate. The five cultivars ranged in final seed size from 270 to 1000 mg (10% H_2O). The number of cells in the two cotyledons varied from 3.2 to 5.4 million.

The results indicated that one of the differences between large and small seeds is the number of cells per seed. Based on correlations between seed size, seed growth rate, and cell number, growth rate seems to be relatively constant on a per cell basis and thus differences in cell number per seed may account for many of the observed differences in seed growth rate, and resultant size.

It also appeared that seed size in Spanish market-type peanuts is restricted by inelasticity of shell, which terminates cell division and prevents cells from attaining full size.

If the growing season is not limiting and the plant can produce more total assimilates in the longer growing season required, yields might be increased by breeding for a longer filling period. This could be done by varying the relationships between pod capacity and seed growth rate, i.e. by selecting for larger pod size, keeping the seed growth rate unchanged or by a slower seed growth rate with no change in pod size.

INTRODUCTION

Although the seed is the economically most important component of peanut (Arachis hypogaea L.) and many other important agricultural crops, the factors which regulate seed growth rate are not well known. The seed is the primary sink for photosynthate during reproductive growth for most crop plants and an understanding of the mechanisms controlling the rate of seed growth is necessary to understand better the yield production process.

Differences in three physiological processes explained most of the yield variation among peanut cultivars: (1) the partitioning of assimilate between vegetative and reproductive parts, (2) the length of the filling period, and (3) the rate of fruit establishment (Duncan et al., 1978).

Studies with other leguminous plants suggest that under good growing conditions the seed growth rate is not a function of the supply of assimilates from the plant, but is largely controlled by the seed or the fruit. With peanuts, the rate of increase in the dry weight of a single fruit is essentially constant under reasonably uniform growing conditions. Only two theories have been advanced to explain why seed growth rates should be nearly constant when the daily assimilate supply available for seed growth is variable. One is that there is something comparable to a valve in the

supply system to the seeds that allows only a limited amount of assimilate to pass. The other is that each growing seed contains approximately the same complement of enzymes to carry on the synthesis required, which would be the case if each seed had the same number of cells. Thus, faster growing seeds would have more cells per seed. If the cells were free to grow to the same final size, seeds with more cells would be expected to be larger when mature.

As a step toward understanding the nature of the differences which underlie variation in seed size and growth rate, the relationship between the number of cells in the seed, seed weight, and seed growth rate was determined for five peanut cultivars.

LITERATURE REVIEW

Plant growth results from division, expansion, and differentiation of cells in localized regions. Although variation in the latter can give rise to differences in the size of an organ, most of the differences in size and general form of an organ arise from differences in number, activity, and persistence of the centers of cell division (Milthorpe and Moorby, 1974).

Seed Size

Seed size varies substantially both between and within different species of plants (Harper et al., 1970; Salisbury, 1974). While the evolutionary and ecological significance of these size variations has been discussed, their cellular basis for the most part is not understood (Davies, 1977).

According to McArthur and Butler (1938), cell number and cell expansion are two separate genetic entities. They considered the basic size factors to be rate genes, one group of which governs the rate or duration of division in the formative stages. Each extra division at this stage doubles the number of cells. On the basis of such differences in cell number, the size increases occurring later by expansion of each of the cells are therefore proportional to the

cell number. They expressed a need for research to discover just how early and by what factors the differences in the number and size of cells comprising the primordium of the embryo are determined; and to demonstrate more precisely by linkage studies the existence and nature of factors more or less specifically controlling cell number and cell size.

Physiological studies have not resolved the relative roles of the sources of photosynthate, of the capacity or strengths of the sinks within the seed, or of the translocation system between them in determining seed size (Evans, 1975). Sink strength and size have not been defined metabolically in terms of enzymatic activities, hormone levels, nucleic acid and protein metabolism, nor physically in terms of cell numbers and cell mass (Dure, 1975).

The average seed size of any one cultivar stays remarkably constant over a wide range of environments compared to seed number. Thus, genetic factors predominate in determining seed size (assimilate deficiency by foliar disease, water deficit during the latter part of the filling period, or early plant death can reduce seed size). Where flowering extends over a long period, seeds develop to much the same size despite a large variation in photosynthate and nutrient supply (Milthorpe and Moorby, 1974).

Hedley and Ambrose (1980) found seed development to be dependent on three levels of plant organization: 1) the maternal influence, 2) the interactions between the embryo and testa and other maternal tissues, and 3) the genotype of

the embryo. They suggested methods to study these effects separately. The use of reciprocal crosses between varieties of contrasting seed size in pea (Pisum sativum L.) allowed the recognition of two systems of control of seed development--an intrinsic one dependent on the seed genotype and the extrinsic control of the maternal environment (Davies, 1975). This phenomenon can be observed in many genera, because the seeds which arise from reciprocal crosses can have completely different phenotypes in spite of having identical genotypes.

Seed Growth Rate

Many observations suggest that the rate of seed growth is controlled primarily by the seed or the fruit and is not simply a function of the supply of assimilate and nutrients from the plant. Boote (1976) reported that the rate of dry weight increase of a single tagged fruit in peanut (Arachis hypogaea L.) is essentially constant under reasonably uniform growing conditions and temperatures. Similar observations were made for soybean (Glycine max (L.) Merrill) showing that the rate of seed growth is insensitive to variations in the supply of assimilate (Egli and Leggett, 1976) and is directly correlated with genetic differences in final seed size (Egli et al., 1978). Koller (1971) found that the seed growth rate in soybean appeared to be controlled primarily by regulatory mechanisms within the seed, rather than by external availability of assimilates. Egli and Wardlaw (1980) cultured soybean cotyledons in vitro with nonlimiting supplies of sucrose and

organic N. They found the seed growth rate to be influenced by temperature and the effects were similar to those observed *in vivo*.

Dreyer et al. (1981) obtained slower growth rates per fruit in peanuts by cooling the soil of the fruiting zone below the average soil temperature. This caused more fruits to be established, which resulted in a higher fruit yield, but a longer filling period was required for the slower-growing seeds. These observations support the hypothesis that the number of fruits established per plant should be inversely proportional to the growth rates of the individual fruits.

Most of the dry weight accumulation in the true seed of the legumes and oil seed crops occurs in the cotyledon tissue (Hsu, 1979) as in soybean and peanut; but the above observations on constancy of seed growth rate, etc. also hold for crops where most of the increase in dry weight during growth of seed occurs as endosperm. This is supported by the observations on wheat (Jenner and Rathjen, 1975, 1977; Sofield et al., 1977) and corn (Poneleit and Egli, 1979).

Egli (1981) summarized the information on the seed growth characteristics of 13 grain crop species from the available literature. These were rice (*Oryza sativa* L.), corn (*Zea mays* L.), wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), sorghum (*Sorghum bicolor* (L.) Moench), pea (*Pisum sativum* L.), field pea (*Pisum arvense* L.), cowpea (*Vigna unguiculata* (L.) Walp), flax (*Linum usitatissimum* L.),

bean (Phaseolus vulgaris L.), broadbean (Vicia faba L.), soybean (Glycine max (L.) Merrill), and peanut (Arachis hypogaea L.). There was a 100-fold variation in seed growth rate among the species and cultivars examined and growth rate was significantly correlated with maximum seed size. The average growth rate for the legumes was considerably higher than that of the small-seeded cereals. The effective filling period (EFP) was less variable than seed growth rate and was not as closely correlated with maximum seed size.

The variations in the length of the EFPs suggested a possibility of increasing yields by selecting for longer EFPs. A relationship between EFP and yield has been shown for soybeans (Hanway and Weber, 1971; Gay et al., 1980), wheat (Evans and Dunstone, 1970), and corn (Daynard et al., 1971; Poneleit and Egli, 1979). This avenue for yield improvement is particularly attractive because, in many environments, crops do not use all of the available growing season in producing their yield, and also because there have been essentially no efforts in the past to use directly this character in plant breeding programs (Egli, 1981).

Cell Number and Size

It has been suggested that differences in seed growth rate are related to the number of cells per seed, assuming growth rate per cell is relatively constant. Although this hypothesis remains unproven, there is evidence in the literature that the main difference among seeds of different sizes is a difference in cell numbers.

Dunstone and Evans (1974) noted no increase in either the specific gravity of the mature grain or endosperm cell size with increase in grain weight during the evolution of wheat (Triticum sp.). Thus, it was presumed that increase in endosperm cell number was the major factor responsible for increase in wheat grain size during evolution.

Brocklehurst (1977) reported that the rate of increase in dry weight of the grain was closely related to the number of endosperm cells found in wheat. Cell number in the endosperm in turn appeared to be regulated by the supply of assimilates available to the grain during the first 2 weeks after anthesis. He also found the difference in grain weight between the cultivars to be closely correlated with the difference in the number of endosperm cells.

Davies (1977) examined twelve species of the genus Vicia and found that seed weight was highly significantly correlated with cotyledon cell numbers. The extremes differed by a factor of 625-fold in dry seed weight; of this variation most (140-fold) was attributable to variation in the number of cells and only a 4.47-fold change was due to differences in cell mass.

Egli et al. (1981) reported that differences in growth rate of soybean seeds were correlated with the number of cells in the cotyledons suggesting that this may be the point of control.

Embryogenesis in angiosperms commences with the double fertilization that gives rise to the zygote (2N) and the triploid cell that will give rise to the endosperm (3N). The embryo itself initially grows very slowly. The endosperm continues to expand in size and cell number, followed somewhat later by the embryo proper, the growth of which is at the expense of the endosperm in dicots. There is no direct vascular connection between the vegetative plant and the embryo itself, rather the vascular tissue coming out of the ovarian tissue ends in the testa/integument tissues of the ovule. The proliferation of the nucellus and/or endosperm results from the nutrient flow from the vegetative plant parts. The extent to which the embryo consumes the nucellus/endosperm during embryogenesis varies among angiosperm species, ranging from total absorption in legumes to almost no absorption in cereals (Dure, 1975).

McKee et al. (1955) made an interesting observation about pea (Pisum sp.) that was subsequently found to hold for other legumes and other dicots as well. He noted that the final number of cells of pea cotyledons is reached less than halfway through the period of seed formation, which is about 50 to 55 days, and long before the cotyledons have accumulated all their starch and protein. Bain and Mercer (1966) divided the development of pea cotyledons into four phases that again emphasized the cessation of cell division in cotyledons early in embryogenesis and prior to the large build-up of the nutritional storage products. Similar observation was made by Millerd and Spencer (1974).

Bils and Howell (1963) observed that cell division in soybean cotyledons ceases about 2 weeks after flowering whereas desiccation does not begin until 50 days. Egli et al. (1981) reported that the estimated maximum number of cells in soybean cotyledons occurred at an average of 24% of the maximum seed dry weight with a range across all cultivars of 18 to 33%.

Loewenberg (1955) noted that the final number of cells in the common bean (Phaseolus vulgaris L.) cotyledons is reached when the dry weight of the embryo is only one-sixth of its final value. This number is reached only 21 days post anthesis whereas seed formation and maturation is not complete until about 44 days. It was also noted by Opik (1968) that cell division in Phaseolus vulgaris and P. coccineus embryos ceases before embryogenesis reaches the halfway point in time and prior to the time of starch and protein deposition in the cotyledons.

Seed development in broadbean (Vicia faba L.) was studied by Millerd et al. (1971). They observed that during cotyledon growth, cell number increased rapidly and essentially the full complement of cells was reached when the cotyledon was about 9 to 10 mm long. After this, only occasional mitotic figures could be seen. The maximum cotyledon length observed was 32 mm.

Growth and Development of Peanut Seed

Seen as a whole, the developmental pattern of legume seed formation is very similar among the species studied (Dure, 1975). For this reason peanuts can be expected to have a seed ontogeny similar to other legumes, though they are unique in some respects. The accumulation of dry weight by a peanut seed follows a pattern consisting of a short phase of exponential growth, a relatively long period of linear growth, followed by a relatively short period of growth at a declining rate. Most of the final dry weight of the seed accumulates during the period of linear growth and the seed reaches maximum dry weight at physiological maturity (McGraw, 1977).

The development of a peanut peg (gynophore) has been described by several researchers (Reed, 1924; Shibuya, 1935; Moore, 1937; Jacobs, 1947; Smith, 1950, 1956a and 1956b; Schenk, 1961). Generally the peg begins rapid geotropic elongation about 1 week after the flower is fertilized and normally begins to penetrate the soil about 8 to 14 days after fertilization. The embryos contain 5 to 27 cells before the pegs penetrate the soil, but development of embryo almost ceases during the time of rapid elongation just before the pegs enter the ground (Schenk, 1961).

Studies by Badami (1935) indicated that the embryo controls elongation and also influences the initiation of fruit enlargement. However, the shell continues to develop when an ovule aborts after enlargement has begun, thus indicating

that the continuation of enlargement is not controlled by the ovules (Gregory et al., 1951).

Ovule development is very slow until the start of fruit enlargement (Badami, 1935; Shibuya, 1935; Smith, 1956; Zamotailov, 1958). There is some disagreement as to time of initiation of rapid embryo development. Shibuya's (1935) results indicate that active embryo development begins during rapid enlargement of the shell. Badami's (1935) observations appear to support this idea. On the other hand, Thevenin's (1953) results indicate that active embryo cell division begins at about the 1-week-old stage of subterranean development and prior to rapid expansion of shell. Smith's (1956b) work indicates embryo development to the 10-celled stage during the first week after fertilization. These discrepancies may not be due to error of one of the investigators, but rather to the fact that they were working with different varieties.

Schenk (1961) kept a chronological record of the development of individual peanut fruits at weekly intervals during the period from entry of the pegs into the soil until the maximum dry matter content was attained. The fruits of Virginia Bunch 67 reached maximum size and fresh weight at 3 weeks after the peg was buried. The inner parenchymatous tissues of the shell then collapsed during the next 2 weeks as the seeds expanded rapidly to fill the shell cavity. After 6 weeks the seed skins collapsed with a resulting rapid decrease in fresh weight. The dry matter content of

the fruit and seed continued to increase for about 5 more weeks. Thus, it took about 11 weeks until the end of maturity.

The Dixie Spanish fruits reached maximum size and fresh weight at 2 weeks and the seed skins collapsed during the fifth to eighth weeks. The dry weight reached maximum at 7 weeks. The developmental period for the Spanish fruits was, therefore, considerably shorter and their maturation more abrupt than the Virginia type. According to Schenk (1961), this abrupt cessation of further development may have been at least partly due to back pressure which presumably built up in the seeds as they became tightly packed in the surrounding and restricting shell.

There are a number of observations to support the hypothesis that the capacity of the enclosing envelope limits seed size. In rice if a small stone or piece of plastic is placed inside the hull at anthesis, at maturity the grain is found to be indented with the shape of the enclosed object (Matsushima, 1957). Similarly the growth of soybean pods and individual seeds within a single pod has been restricted by appropriate bandaging; and in two-seeded peanut pods, when one of the seeds fails to develop, the remaining seed may grow much larger than if the two seeds had grown normally (Duncan et al., 1979).

Technique

The cell-counting technique utilized in this experiment was devised originally by Brown and Rickless (1949) for

determining the total and the meristematic cell numbers in a root meristem. It involved macerating the tissue in a known volume of fluid, and subsequently determining the appropriate number of cells in an aliquot on a haemacytometer slide. Five percent chromic acid was used as a macerating agent, and the cells were dispersed by shaking the solution.

Rijven and Wardlaw (1966) modified this method so that it was satisfactory for determining cell numbers in developing endosperm tissue. The tissue was stained in bulk in Feulgen's reagent, and then macerated with fungal cellulase. Counts were made from the resulting nuclear suspension to determine cell numbers.

MATERIALS AND METHODS

Five peanut (Arachis hypogaea L.) cultivars known to be genetically different in final seed size were grown until mature in 25-cm diameter pots filled with 75:25 soil-vermiculite mixture. The soil used was a fine sandy loam (taxonomic order unknown) from the vicinity of Tifton, Georgia, known to support high peanut yields. The pH was 6.5. The cultivars are listed in Table 1. The pod and seed size are compared in Fig. 1.

The experiment was conducted during April through August of 1979 and 1980 at the University of Florida Agronomy Farm. The soil was not inoculated, but fertilized with 3 g of 2-10-10 fertilizer/pot. Gypsum ($CaSO_4 \cdot 2H_2O$) was applied at the rate of 2 g/pot during pegging. Pesticides listed in Table 2 were applied at recommended rates. The average maximum and minimum temperatures during the experimental period are reported in Table 3. Seeds were harvested at weekly intervals after pegging until maturity. Fresh weights were measured with an analytical precision balance. Most of the seeds were fixed in a 3:1 absolute alcohol-glacial acetic acid mixture after the removal of seed coats. After being in the fixative for at least 4 hours the seeds were stored in 70% ethanol. Remaining seeds were dried in a 65 C oven and weighed. These dry weights were used to establish a relationship with fresh weights.

Table 1

Characteristics of cultivars used in the experiment

Cultivar	Subspecies	Variety	Market type	Days to first flower	Days to maturity	Average final seed weight (10% H_2O) (mg)
'Florigiant'	hypogaea	hypogaea	Virginia	30-35	135-140	1000
'Florunner'	hypogaea	hypogaea	Runner	30-35	130-135	700
'Starr'	fastigiata	vulgaris	Spanish	30-35	125-130	400
'Krinkle'	fastigiata	vulgaris	Spanish	25-30	115-120	300
'Chico'	fastigiata	vulgaris	Spanish	25-30	95-100	270

Source: Sources of data for days to maturity in Table 1 are as follows:
'Florigiant', 'Florunner', and 'Starr': (McGill, J.F., 1974).
'Krinkle': (Godoy, I.J., 1981).
'Chico': (Dashiell, K., 1981).

Figure 1. Comparison of pod and seed sizes of the five cultivars

Table 2

Chemicals used for pest control on plants in pots

Trade name	Common name	Chemical name	Use
Arasan	Thiram	tetramethylthioperoxydicarbonic diamide	seed treatment (fungicide)
Kelthane	Dicofol	4-chloro-α-(4-Chlorophenyl)-α-(trichloromethyl) benzenemethanol	acaricide
Cygon	Dimethoate	0,0-dimethyl S-[2-(methylamino)-2-oxoethyl] phosphorodithioate	acaricide and insecticide
Bravo	Chlorthalonil	2,4,5,6 - tetrachloro -1,3 - benzenedicarbonitrile	fungicide

Table 3

Average maximum and minimum monthly temperatures
for 1979 and 1980 experimental periods

Month	Maximum temperature (°C)		Minimum temperature (°C)	
	1979	1980	1979	1980
April	28.1	27.3	15.1	12.7
May	30.0	30.5	16.9	17.4
June	32.6	33.0	20.0	20.2
July	34.0	34.0	22.6	22.4
August	33.0	34.0	21.7	22.4

Cell Counting

The number of cells in the cotyledons was measured following the method of Rijven and Wardlaw (1966). First the seeds were hydrated by placing in 50% and then 30% ethanol for 15 minutes each, followed by 30 minutes in distilled water. This hydration was followed by hydrolysis with 5N hydrochloric acid (HCl) while being held for 45 minutes in a cold ice bath, 15 minutes at 60 C and 60 minutes at 20 C. The seeds were rinsed by placing in distilled water for 2 hours. To stain, the seeds were placed in tightly stoppered bottles with Feulgen's reagent (Appendix 1-A) for 4 to 8 hours. The stained seeds were washed in distilled water for 6 hours to overnight. Water was changed several times to remove excess reagent.

The seed tissue was macerated by placing the cotyledons in 0.5% cellulase (1,4 - [1,3; 1,4] - β - D - Glucan 4 - glucanohydrolase) from Aspergillus niger at 40 C and pH 5.0 (Citrate-phosphate buffer--Appendix 1-B). The preparation was occasionally shaken throughout the digestion period which varied from 2 to 8 hours depending on the age of the seed. In very young seeds, the hydrolysis in HCl alone gave an adequate cell separation, making maceration in cellulase unnecessary. The cells were dispersed by forcing the material through a syringe several times with a relatively small needle. The cell solution was brought to a known volume. A 1-ml aliquot was evenly dispersed in about 5 ml of water in the tube of a filtration apparatus, and drawn by vacuum onto a

plain white porous membrane filter, 25 mm in diameter and 0.45 μ pore size. The filter was mounted on a slide in 50% glycerol.

Cells of the epidermal layer and the embryonic axis were not counted for two main reasons:

1) There are many small cells from these two tissues which remain in aggregates, and cannot be properly separated (Fig. 2).
2) Cotyledons make up the bulk of the peanut embryo.

The counts were made under a stereo-microscope. Twenty fields were counted for each sample and four such samples were prepared per cotyledon.

The number of cells per cotyledon was determined using the following formula:

$$\text{cells/cotyledon} = \frac{\text{area of precipitation} \times \text{number of cells counted} \times \text{D.F.}}{\text{area counted}}$$

where,

$$\text{area of precipitation} = \pi \left(\frac{\text{diameter of filter}}{2}\right)^2$$

$$= \pi \left(\frac{21}{2}\right)^2 = 346.36 \text{ mm}^2$$

$$\text{area counted} = \pi \left(\frac{\text{diameter of field}}{2}\right)^2$$

$$= \pi \left(\frac{1.50}{2}\right)^2 = 1.7671 \text{ mm}^2$$

$$\text{D.F.} = \text{dilution factor} = \frac{\text{total volume}}{\text{volume counted}}$$

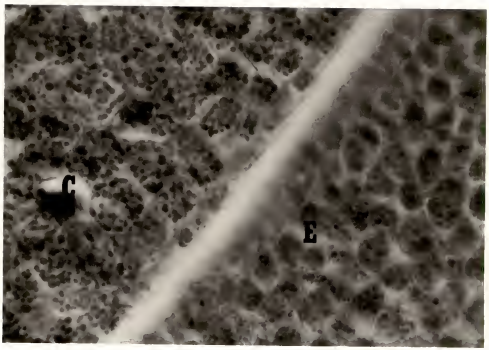


Figure 2. Transverse section of 'Krinkle' seed showing cotyledonary (C) and embryo (E) cells

number of cells counted = average of four slides

$$\text{cells/cotyledon} = \frac{346.36}{1.7671} \times \text{dilution factor} \times \text{number of cells counted}$$

$$= 196.00475 \times \text{dilution factor} \times \text{number of cells counted}$$

Data were analyzed using the Statistical Analysis System (SAS) by Barr et al. (1979). Computing was done using the facilities of the Northeast Regional Data Center of the State University System of Florida, located on the campus of the University of Florida in Gainesville.

Sectioning

Mature seeds of all five cultivars were transversely sectioned with a cryostat-microtome to determine if any differences existed betwen them as far as cell size is concerned. The sections were stained with a safranin-fast green combination. Safranin stained the cell walls red and fast green stained the cytoplasm bluish green. The thickness of the sections was 30 μ. The seeds used for sectioning were the ones previously stored in 70% ethanol.

Tagging

Pegs were tagged and harvested weekly and dry weights taken of the seeds. This was used to calculate the seed growth rate and the effective filling period. Since the experiment had to be terminated prematurely because of damage by wild animals, the data are approximate.

RESULTS AND DISCUSSION

The five cultivars 'Florigiant', 'Florunner', 'Starr', 'Krinkle', and 'Chico' differed in the final cell number in the cotyledons (Table 4). These five cultivars fell into 3 categories which showed a statistically significant difference in the maximum cell number ($\alpha = .05$). The categories were:

1) Large seeded--'Florigiant' and 'Florunner'

2) Medium--'Starr'

3) Small seeded--'Krinkle' and 'Chico'.

No statistically significant difference was observed between 'Florigiant' and 'Florunner' as well as between 'Krinkle' and 'Chico'.

Since it is very difficult to count the cells in the cotyledons of dry seeds, the cell numbers obtained were related to the seed fresh weight (Figs. 3 through 7). A linear regression curve fitted to the data of fresh weight of seed against dry weight for the five cultivars gave a high coefficient of determination ($r^2 > 0.95$). For this reason it was thought appropriate to relate the cell count reported in this study to the seed fresh weight.

Ideally the cell number should have been related to seed age and an attempt was made to tag the pegs, but due to the extreme variability encountered, fresh weight was chosen instead. Dure (1975) stated that "it is relatively

Table 4

Cell numbers for the five cultivars

Seed fresh weight (mg)	Cultivars				
	'Chico'	'Krinkle'	'Starr'	'Florunner'	'Florigiant'
	Average Cell Number (x 10^6)				
0- 50	0.88	0.94	0.42	0.73	0.81
51- 100	1.43	1.33	-	1.42	1.70
101- 150	2.06	0.61	2.31	-	2.60
151- 200	1.98	2.39	1.34	2.74	-
201- 250	2.56	2.66	2.49	3.29	2.74
251- 300	2.67	2.72	3.44	4.89	3.23
301- 350	2.83	2.66	3.36	2.96	3.52
351- 400	2.97	3.00	4.13	4.12	4.40
401- 450	3.11	3.23	4.33	4.73	4.32
451- 500	3.52	3.29	4.21	4.06	4.36
501- 550		3.69	-	5.54	4.88
551- 600		3.61	5.15	5.13	4.93
601- 650			3.87	4.21	5.64
651- 700			4.71	-	5.78
701- 750			4.55	4.75	3.07
751- 800			4.34	4.51	-
801- 850			4.82	5.46	5.01
851- 900			3.99	5.49	-
901- 950				6.21	6.24
951-1000				-	5.53
1001-1050				6.32	-
1051-1100					4.51
1201-1250					4.99
1301-1350					5.07
1401-1450					5.98
1551-1600					5.69
1601-1650					5.56

Figure 3. Relationship between fresh and dry weights of 'Florigiant' cotyledons

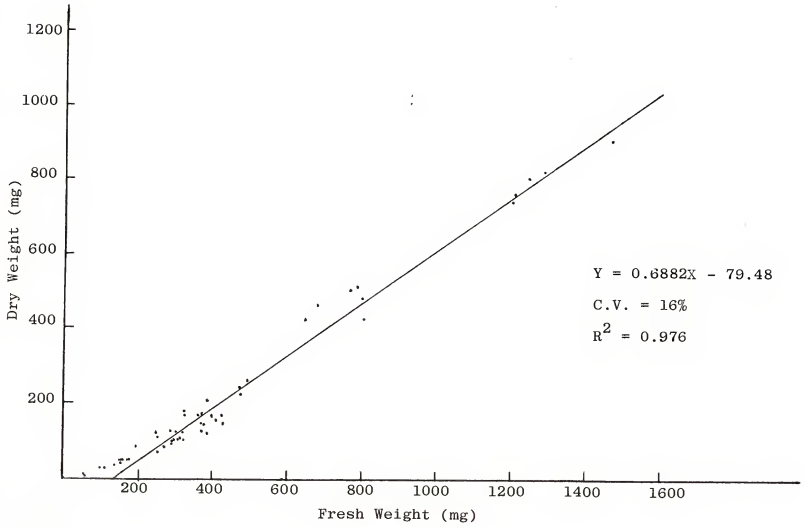

1200
1000
800
600
400
200
Dry Weight (mg)
200
400
600
800
1000
1200
1400
1600
Fresh Weight (mg)
Y = 0.6882X - 79.48
C.V. = 16%
R^2 = 0.976

Figure 4. Relationship between fresh and dry weights of 'Florunner' cotyledons

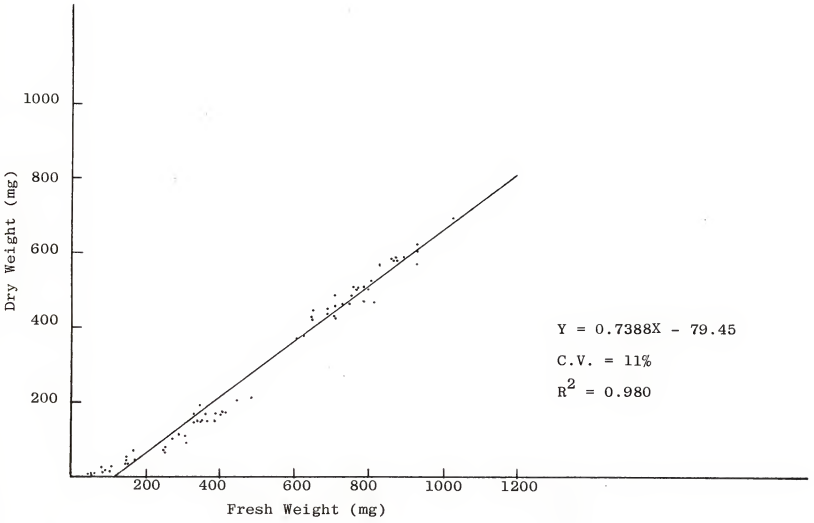
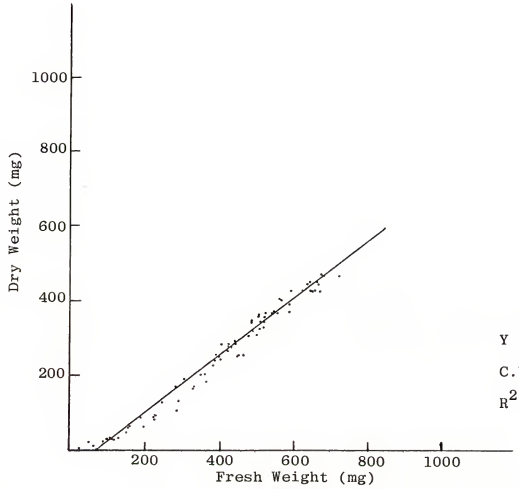

1000
800
600
400
200
Dry Weight (mg)
200
400
600
800
1000
1200
Fresh Weight (mg)
Y = 0.7388X - 79.45
C.V. = 11%
R^2 = 0.980

Figure 5. Relationship between fresh and dry weights of 'Starr' cotyledons



$Y = 0.7681X - 59.00$

C.V. = 8%

$R^2 = 0.980$

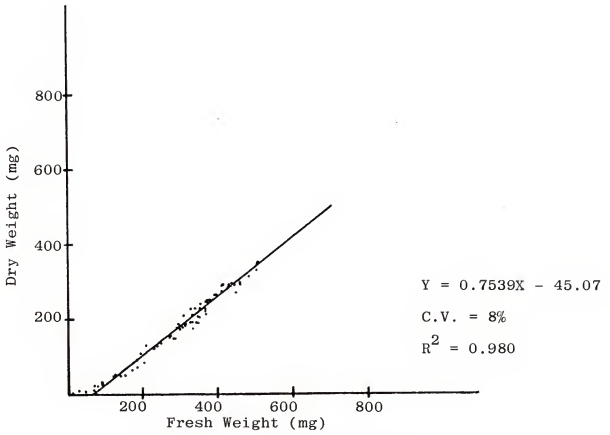


Figure 6. Relationship between fresh and dry weights of 'Krinkle' cotyledons

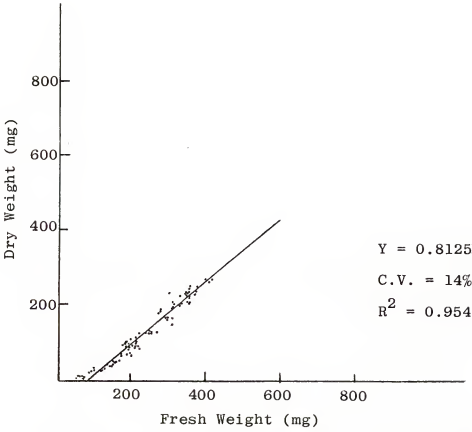


Figure 7. Relationship between fresh and dry weights of 'Chico' cotyledons

easy to obtain developing material all of which is at precisely the same developmental stage. All that is necessary is to tag flowers at the time of anthesis and harvest the pods, bolls, etc. when necessary" (p. 276). This may be true in the case of most plants but in peanuts, anthesis to beginning of pod development varies widely. Also the underground pod development made it difficult to mark the time at which the peg-swelling began. Boote (1976) noted that early-set fruits were larger in size and heavier than later-set fruits, which may also cause some variability. Whenever the plant is disturbed, all the leaves close and may remain so for several hours, possibly affecting the growth processes. Tagging and burying of pegs and cutting off the extra pegs may also result in disturbing the normal processes of peg development.

The maximum number of cells in the cotyledons of 'Florigiant', 'Florunner', 'Starr', 'Krinkle', and 'Chico' were approximately 5.4, 5.4, 4.8, 3.3, and 3.2 million, respectively, as predicted by the regression equation. A non-linear regression program was used with the exponential equation $Y = A(e^{-B/X})$ giving a satisfactory fit for the data of seed fresh weight plotted against the cotyledon cell number (Figs. 8 through 12). Here Y = Cell number, X = Seed fresh weight and A,B = Parameters (A = maximum cell number; B = fresh weight at which cell number = 0.36A).

Figure 8. Relationship between cell number and fresh weight of 'Florigiant' cotyledons

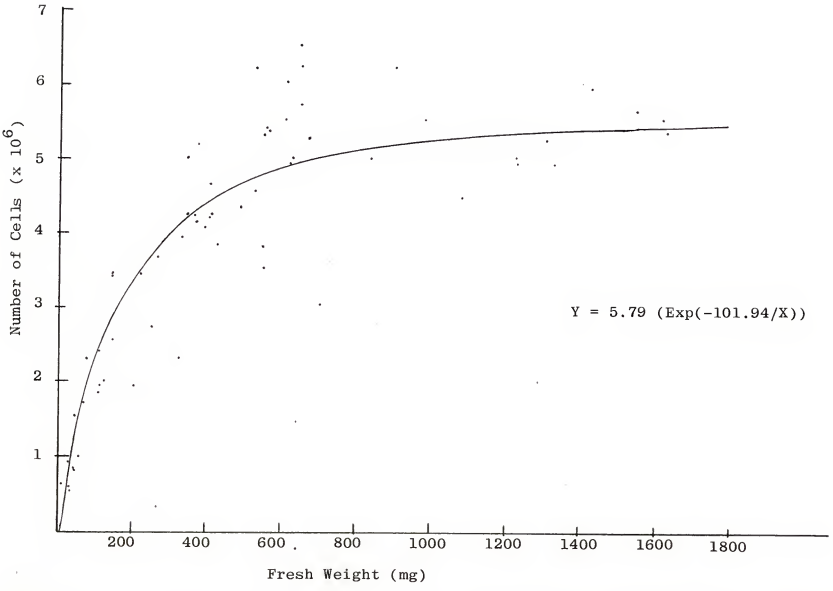
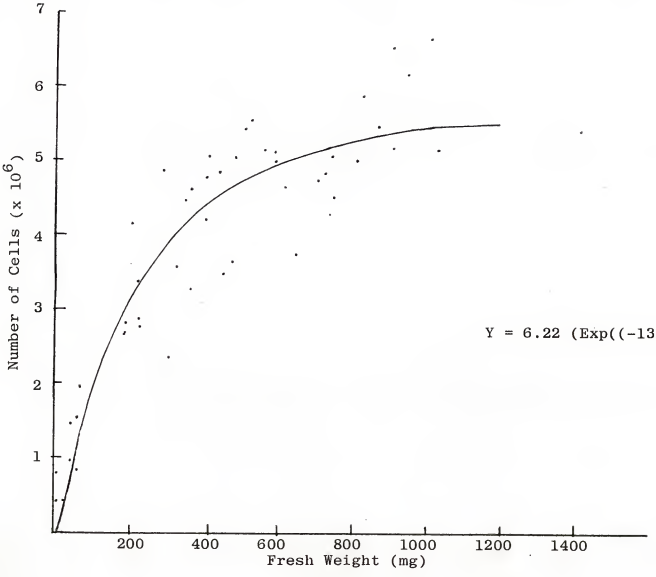

Number of Cells (x 10^6)
7
6
5
4
3
2
1
200
400
600
800
1000
1200
1400
1600
1800
Fresh Weight (mg)
Y = 5.79 (Exp(-101.94/X))

Figure 9. Relationship between cell number and fresh weight of 'Florunner' cotyledons


Number of Cells (x 10^6)
7
6
5
4
3
2
1
200
400
600
800
1000
1200
1400
Fresh Weight (mg)
Y = 6.22 (Exp((-132.31/X))

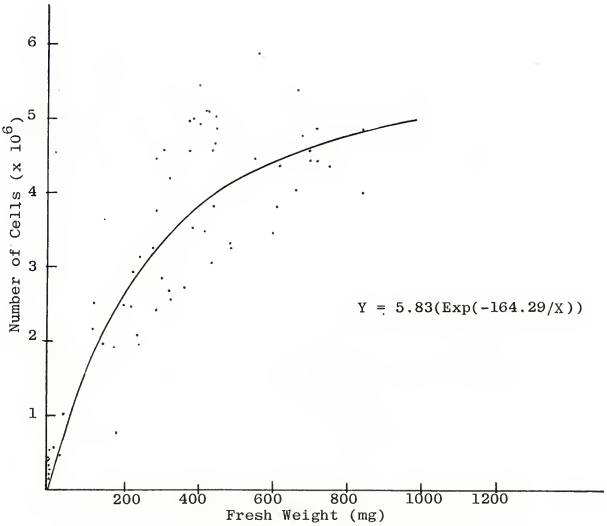


Figure 10. Relationship between cell number and fresh weight of 'Starr' cotyledons

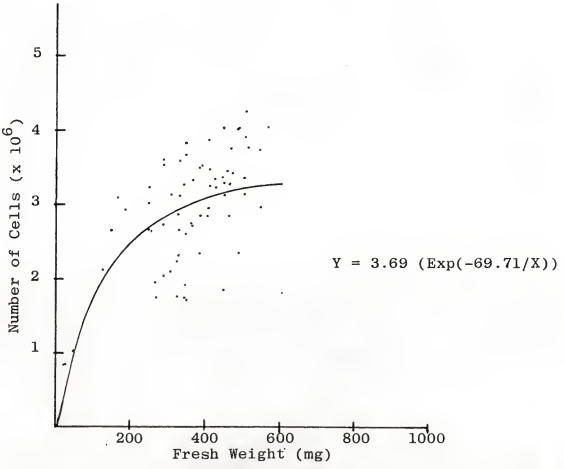


Figure 11. Relationship between cell number and fresh weight of 'Krinkle' cotyledons

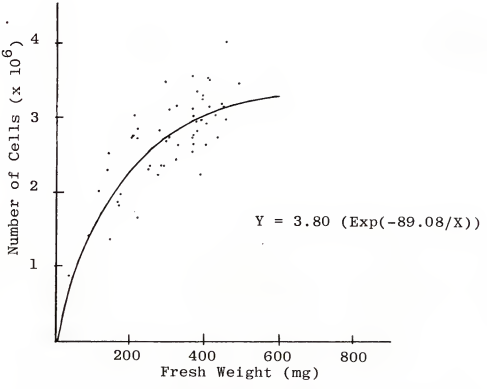


Figure 12. Relationship between cell number and fresh weight of 'Chico' cotyledons

One-half of the final cell number for the three Spanish-type peanuts was reached at about 25% of the final fresh weight. The corresponding figures for 'Florigiant' and 'Florunner' were 12 and 20%, respectively (Table 4). In terms of dry weight, this cell number was reached at < 1% in 'Florigiant' and at 25% for 'Starr'. For the remaining three varieties, half of the estimated maximum cell number was reached at 6 to 8% of the final dry weight.

None of the cultivars showed a definite plateauing of cell number, though the increase in number towards maturity was very slight especially for 'Florigiant'. This indicated that cell division continued until maturity, though at a decreasing rate. Interestingly, in all five cultivars the very active cell division appeared to terminate at about 600 mg seed fresh weight. The much more active cell division continuing until maturity in Spanish types may have been due to the premature termination of seed growth by pod wall restriction.

Peanut yields under good management have continued to increase over the last 30 years, but the thought that a limit may be near has focused the attention of scientists on theoretical maximum yields, which are affected by partitioning, photosynthetic rate, filling period duration, and early season establishment, translocation and nutrient accumulation. In the modern peanut cultivars, yield is not limited by a lack of potential fruit to fill, because many more flowers and pegs are produced than mature pods. The rate of photosynthesis for a single leaf is among the best for C_3 plants

and there does not appear to be much room for improvement of canopy photosynthesis. Full ground cover is reached before the filling period gets well underway. Prior accumulation and later re-mobilization of nutrients is less well known than for other legumes such as soybean. Thus, for any appreciable increase in theoretical maximum peanut yields, lengthening of filling period appears to be the most promising area.

For a whole plant with many seeds, the length of the filling period can be defined as extending from the dry weight increase of the first fertilized ovule until the last developing seed reaches its maximum dry weight. The aim of extending the filling period thus has two aspects; extending it for the whole plant or extending it for a single seed. Extending filling period for the whole plant may not be a very practical approach due to considerations such as harvesting. If later-set pods are allowed to mature, a loss of earlier-set pods can result. Extending the filling period of individual seeds does not present such problems, at least to as large an extent as in previous case. It could result from slower-growing seeds or larger seed with no increase in growth rate or any combination of the two. Slow growth rate of individual seeds would allow more seeds to be initiated and filled, hence giving a higher seed yield, but a longer filling period would be required.

The time required for an individual seed to reach maximum size when growing at its constant rate is defined as effective filling period (EFP), calculated by dividing the maximum seed size by the constant rate of seed growth.

The number of cells in peanut cotyledons is 100-fold greater than the number in the wheat endosperm, and larger by a factor of 10 than the number of cells in pea cotyledons and endosperm of corn kernels (Table 5). It is similar to those reported for broadbean, soybean, and bean. The relations of cell numbers to the average seed size, seed growth rate, and EFP reported in the literature and compiled by Egli (1980) are given in Table 5.

Both seed size and growth rate appear to be positively correlated with cell number (Fig. 13), but not the EFP. As can be expected, seed growth rate was closely correlated to seed size in most cases. Broadbean seeds were large in size and had a high growth rate in spite of having a small cell number in the cotyledons. Bean seeds were small in size and had a small cell number, but a relatively faster seed growth rate. In spite of having the highest cell number in the cotyledons, soybean had a very slow seed growth rate. This may be because soybeans are fairly unique in requiring the greatest amount of N in seed production and being one of the lowest producers of seed biomass per unit of photosynthate (Sinclair and De Wit, 1975). Also peanut and soybean seeds are different from those of other crops in having a high lipid content.

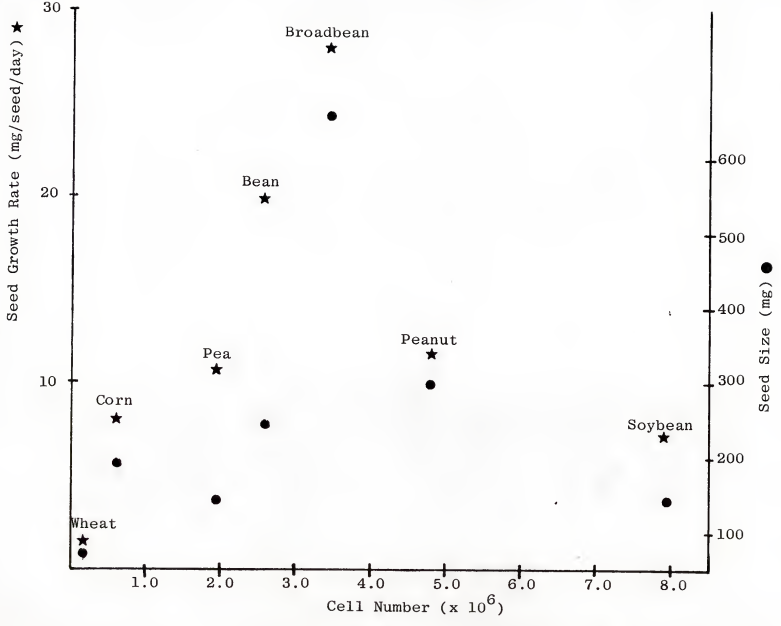
Table 5

Seed growth characteristics and number of cells in legume seed cotyledons and cereal seed endosperm

Species	Mean rate	Mean EFP	Mean maximum size	Cell number	Sources of data for cell number
	(mg/seed/day)	(days)	(mg/seed)	(x 10^6)	
Wheat (Triticum aestivum L.)	1.5	28	40	0.054-0.065 0.122-0.158	Wardlaw, 1970 Brocklehurst, 1977
Corn (Zea mays L.)	8.1	34	280	0.3-0.6	Egli, Priv. Comm.
Pea (Pisum sativum L.)	10.5	22	195	0.24 1.4 1.26-1.97	Millerd & Spencer, 1974; McKee et al., 1955; Davies, 1975
Bean (Phaseolus vulgaris L.)	19.8	18	345	2.6	Loewenberg, 1955
Soybean (Glycine max (L.) Merr.)	6.9	28	194	5.7-10.2	Egli et al., 1981
Broadbean (Vicia faba L.)	27.8	39	1216	0.025-3.48 2.8	Davies, 1977 Millerd et al., 1971
Peanut (Arachis hypogaea L.) Spanish Type	11.6	43	500	4.8	

Source: Sources of data for seed growth characteristics in Table 4 are as follows: Wheat: (Asana and Williams, 1965; Kapoor and Heiner, 1976; Agrawal and Kaurr, 1977; Jenner and Rathjen, 1977; Sofield et al., 1977). Corn: (Groszman and Sprague, 1948; Duncan et al., 1965; Carter and Poneleit, 1973; Tollenaar and Daynard, 1978; Poneleit and Egli, 1979). Pea: (Danielson, 1952, 1956; Robertson et al., 1962; Millerd and Spencer, 1974; Harvey and Keely, 1976). Bean: (Lowewenberg, 1955; Kloz et al., 1966; Opik, 1968; Hsu, 1979). Soybean: (Garner et al., 1914; Ohmura and Howell, 1962; Egli, 1975; Egli et al., 1978). Broadbean: (Briaty et al., 1969; Ishag, 1973). Peanut: (Pickett, 1950).

Figure 13. Plot of seed growth rate and seed size against the cell number in the seed of seven crop species


Seed Growth Rate (mg/seed/day) ★
30
20
10
Broadbean
Bean
Pea
Peanut
Corn
Soybean
Wheat
600
500
400
300
200
100
Seed Size (mg) ●
1.0
2.0
3.0
4.0
5.0
6.0
7.0
8.0
Cell Number (x 10^6)

Many of the discrepancies in the above data may be due to techniques and to the variety of environments in which the data were collected, since differences in temperature can cause large differences in seed growth rate. Also there may be inaccuracies involved in estimating seed growth rates indirectly from available data. The data on seed size, seed growth rate, EFP, and cell number in most instances were not available from the same source. According to Egli (1981), there was a large amount of variation in EFP within a species, implying that selection for longer EFPs may be an important way of increasing crop yields. This is assuming that there are no abnormal conditions limiting the filling period and that the plant can produce more total assimilates in a longer growing season and more seeds can be initiated and filled.

The characteristics of the growth and development of the individual seed may play an important role in determining sink activity and the ultimate yield. Each plant partitions assimilates between reproductive and vegetative growth in proportions determined chiefly by the plant's genetic background. It has been suggested that the number of seeds per plant is fixed at a level where the total sink demand, as determined by seed number and seed growth rate, is in balance with the available assimilate supply partitioned to it. Peanuts rarely abort pods once they start growing and the pod load is established by a progressive addition indeterminately until a final seed number is reached. Thus

the seed number is established as determined by the demand per seed (Duncan and McCloud, 1980). This hypothesis is supported by the observations of Dreyer et al. (1981). They could increase the number of pods produced per unit area in peanuts by slowing the rate of growth of individual pods. Yield was correspondingly enhanced, but additional time was needed for the slower-growing pods to attain their mature weight. Of course this additional time is not always available due to lack of water, pest problems, etc.

These data show that one of the differences between large and small seeds in peanuts is the number of cells per seed. Thus, it is interesting to speculate that cell growth rate may be relatively constant and differences in cell number per seed may account for much of the observed difference in seed growth rate. Also, assimilate demand per seed could be related to the cell number in the cotyledons. This suggests that changes in seed yield might be effected by breeding for seeds with larger cells that take a longer time to develop. This would require a longer filling period.

Contrary to all the reports in the literature on wheat, corn, pea, bean, soybean and broadbean, cell division in peanut cotyledons did not appear to be completed early in seed development. One-half of the cell number was reached by the end of 3 weeks in 'Florigiant', 'Krinkle' and 'Chico' and by the end of 4 weeks in 'Starr' and 'Florunner' (Figs. 3 through 7 and Tables 4 and 6). This suggests that something in addition to number of cells in cotyledons controls the rate of seed growth during the linear phase.

Table 6

Average dry weight increase in seeds

Weeks	Cultivars				
	'Chico'	'Krinkle'	'Starr'	'Florunner'	'Florigiant'
	Seed Dry Weight (mg)				
1	2	-	-	-	-
2	8	3	-	1	3
3	50	8	5	10	12
4	100	84	23	26	71
5	183	126	91	81	230
6	-	151	184	166	-
7	-	-	-	208	-
8	280	192	-	298	-
9	-	-	195	-	-

In peanuts the ovary starts growing immediately upon fertilization and the pod has nearly attained its full size and weight by the time the ovules begin to grow rapidly (Duncan et al., 1979). During pod growth, the pericarp composed of fiber and sclerenchyma cells thickens soon after seed development begins, so there appears to be limited possibility of stretching. There are a number of observations to support the hypothesis that the capacity of the enclosing envelope limits seed size in natural environment, at least to a degree (Matsushima, 1957; Duncan et al., 1979). Godoy and Norden (1981) reported negative correlations between fruit density and fruit volume in peanuts. This suggested that seeds grown inside pods with genetically smaller cavities may be compacted by the shell, in the cultivars studied.

A number of pods were observed in which one seed had ceased to grow early in its development and the remaining seed had grown to fill both cavities. Seeds of the Spanish types especially were compressed and the pressure exerted by the growing seeds caused darkening of the inside of the pods. In the case of 'Florigiant' and 'Florunner', however, there was only a slight distortion of seeds and little darkening of the inside of the pods. In these cases seed weight may be restricted by the testa. It thus seems reasonable to assume that seed weight in many but not all peanut cultivars is restricted by the capacity of the pods.

One of the reasons for higher pod and seed yields in 'Florigiant' and 'Florunner' as compared to the Spanish cultivars may be attributed to the length of the filling period. There was up to a 40-day difference in days to maturity between 'Chico' and 'Florigiant', but days to first flower did not differ much (Table 1). The average yields produced are reflected by the days to maturity, except that 'Florunner' produces higher yields than 'Florigiant'. Since the filling period for 'Florigiant' and 'Florunner' is longer, there is enough time for the cell division to be more or less completed and for the cells to expand and attain their maximum size. This is because seed growth does not appear to be restricted prematurely by the pods.

In the Spanish types, since cell division continued until maturity, the cells probably did not achieve maximum size. The reason for this may have been limiting pod capacity. The pods were small and the seeds continued their growth and development until the pod was tightly filled. When no more room was left for expansion, cell division and expansion ceased. This was also borne out by observing sections of cotyledons. Cells in the cotyledons of Spanish cultivars were smaller and had thinner cell walls than those of 'Florigiant' and 'Florunner' (Fig. 14). Average area of the cells measured by a compensating polar planimeter was approximately 6.6×10^{-5} cm^2 for 'Chico' and 13.5×10^{-5} cm^2 for 'Florigiant'. The small-seeded Spanish types had more

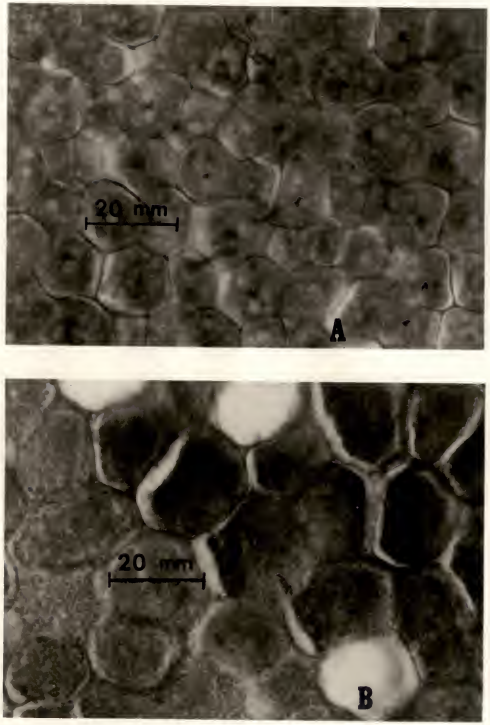


Figure 14. Comparison of cell size between transverse sections of 'Chico' (A) and 'Florigiant' (B) cotyledons (x 320)

cells per unit of seed weight (11,500 cells/mg) than 'Florigiant' (5,400 cells/mg) and 'Florunner' (7,700 cells/mg). This further supports the hypothesis that growth of the Spanish-type seeds was stopped prematurely by pod wall restriction.

Seed growth rates for the five cultivars were estimated using the dry weight of seeds from tagged pods (Table 6 and Fig. 15). The seed growth rate data were used for approximating EFP (Table 7). The maximum estimated cell number was positively correlated with final seed size ($r = 0.970$) and seed growth rate ($r = 0.925$). 'Krinkle' had the lowest seed growth rate, but 'Chico' had the shortest EFP. 'Florigiant' had the highest seed growth rate and longest EFP, because of the very large seed size.

Figure 15. Increase in dry weight of seeds of developing peanut pods

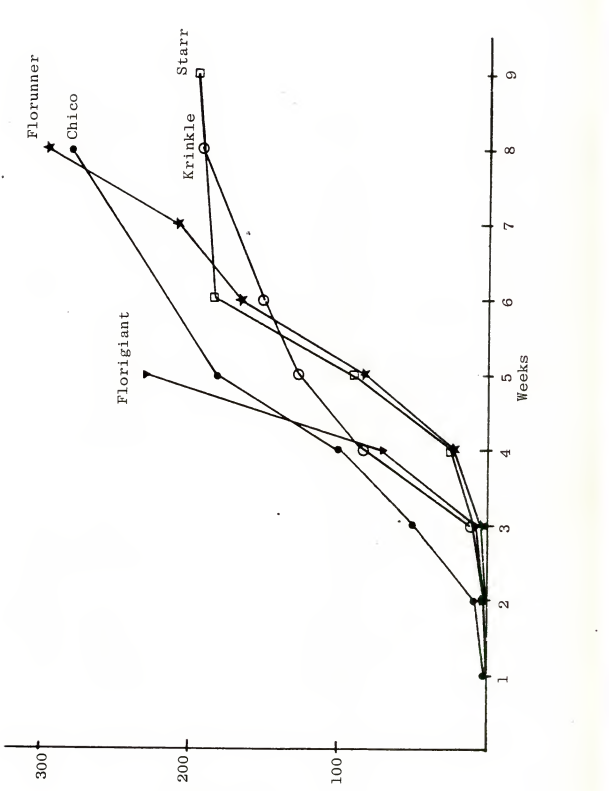

Florunner
Chico
Krinkle Starr
Florigiant
300
200
100
Weight (mg)
1
2
3
4
5
6
7
8
9
Weeks

Table 7

Seed growth characteristics of the five cultivars

Cultivar	Estimated seed growth rate	Maximum cell no.	Effective filling period	Final seed size
	(mg/seed/day)	(x 10^6)	(days)	(mg)
'Florigiant'	14	5.4	70	1000
'Florunner'	12	5.4	58	700
'Starr'	9	4.8	44	400
'Krinkle'	6	3.3	50	300
'Chico'	7	3.2	38	270

SUMMARY AND CONCLUSIONS

During the 1979 and 1980 growing seasons a greenhouse study was conducted to relate seed size and seed growth rate to cell number in peanut cotyledons. The five cultivars ranged in final seed size from 270 mg for 'Chico' to 1000 mg for 'Florigiant'. The number of cells in the cotyledons ranged from 3.2 to 5.4 million.

Except for 'Florigiant' the seeds of four other cultivars studied are quite unlike seeds of other crop plants, which are reported to reach the maximum cell number early in their development.

1) Cell number continued to increase until maximum fresh weight was reached in seeds of 'Starr', 'Krinkle', and 'Chico' cultivars.
2) Cell number increased in seeds of 'Florigiant' cultivar, only until they reached approximately one-third of their final fresh weight.
3) Cell number in seeds of 'Florunner' cultivar apparently stopped increasing at about maximum fresh weight, but appear to be intermediate between 'Florigiant' and the Spanish types.

Two observations in the experiment support the hypothesis that the final seed weight of some peanut cultivars is limited by the volumetric capacity of the pod.

1) The increase in cell number of the seeds of Spanish cultivars continued until they reached maximum fresh weight.
2) The cells of the seeds of Spanish cultivars were smaller than those of Virginia and runner types at maximum fresh weight.

Both observations are consistent with the idea that growth was arrested prematurely by pod-wall restraints. This would imply that 'Florigiant' pods were large enough and so did not stop seed growth. As a result,

a) 'Florigiant' seeds were larger
b) 'Florigiant' had a longer EFP
c) Cells of the 'Florigiant' seeds were larger
d) Increase in cell number ceased when seeds reached one-third final fresh weight.

The initial assumption that seed growth rate should be related to cell number is supported by the positive correlation between seed growth rate and cell number ($r = 0.925$). Seed growth rate was also related to seed size ($r = 0.975$).

Most research on peanuts so far implies that the major part of the increase in potential yield through breeding has come from improving the percentage of assimilate partitioned to pods and seeds. As partitioning approaches 100%, as it has in the latest releases, the possibility of further increases by increasing partitioning factor obviously decreases. If the growing season is limiting and the plant can produce more

total assimilates in the longer growing season required, yields might be increased by breeding for a longer filling period; or by treatment with growth regulating chemicals.

Study of cell number in seeds is important, because it involves one aspect of yield determination, viz., rate of seed growth, which in turn determines seed number and filling period duration.

APPENDIX

1-A: Feulgen's reagent (Darlington and LaCour, 1976):

Dissolve 1 g basic fuchsin by pouring over it 200 ml boiling distilled water.

Shake well and cool to 50 C.

Filter and add 300 ml 1N HCl to filtrate.

Add 3 g potassium meta bisulfite ($K_2S_2O_5$).

Allow solution to bleach for 24 hours in a tight-stoppered bottle, in the dark.

Add 0.5 g decolorized carbon.

Shake well for about a minute and filter rapidly through coarse filter paper.

Store in a tightly stoppered bottle in the dark at 4 C.

1-B: Citrate-phosphate buffer:

10.3 ml 0.2M Na_2HPO_4 + 9.7 ml 0.1M citric acid

Adjust to pH 5.0.

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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